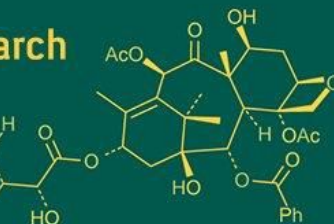
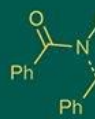
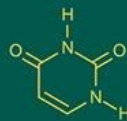
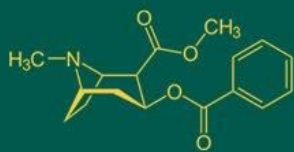


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Screening of advanced biofortified rice lines for resistance to brown planthopper (*Nilaparvata lugens*) using standard seed box technique

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Abstract

Brown planthopper (BPH), *Nilaparvata lugens* (Stål), is one of the most destructive insect pests of rice, causing severe yield losses. The present study was undertaken to evaluate advanced biofortified rice lines for resistance to BPH using the standard seed box screening technique under glasshouse conditions. A total of 30 advanced biofortified lines were tested along with resistant check Ptb33 and susceptible check TN1. The average damage scores across the test entries ranged from 1.8 to 9.0, with Ptb33 recording a score of 1.7 and TN1 showing 9.0. Based on damage ratings, five lines (NQ-BIO 8, NQ-BIO 10, NQ-BIO 26, NQ-BIO 9, and NQ-BIO 21) were classified as resistant, with scores between 1.8 and 2.9. Another five lines (NQ-BIO 6, NQ-BIO 7, NQ-BIO 15, NQ-BIO 24, and NQ-BIO 25) were moderately resistant (3.2–5.0), while four lines (NQ-BIO 1, NQ-BIO 22, NQ-BIO 27, and NQ-BIO 17) were moderately susceptible (5.2–6.9). The remaining 16 lines were identified as susceptible or highly susceptible, showing scores between 7.3 and 9.0. These findings reveal the existence of promising resistant and moderately resistant biofortified lines, which can serve as potential donors for breeding programs aimed at developing durable BPH-resistant rice varieties.

Keywords: Screening, rice, biofortified lines, brown planthopper, resistance, standard seed box technique

Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop and a staple food for more than half of the world's population. Globally, rice provides 19% of dietary energy and 13% of protein requirements, highlighting its central role in food and nutritional security. Asia alone accounts for nearly 90% of global rice production and consumption, with India being the second-largest producer after China. Within India, rice is cultivated on more than 44 million hectares, producing around 130 million tonnes annually (FAO, 2024). The crop is uniquely adapted to diverse agro-ecological zones ranging from irrigated lowlands to upland rainfed systems, underscoring its resilience and socio-economic importance.

In India, rice is not only a staple food but also a key component of the rural economy. Among the rice-growing states, Chhattisgarh, popularly called the "rice bowl of India" plays a crucial role. The state has consistently ranked among the top six rice-producing states, contributing significantly to the central pool of food grains. During the 2023–24 crop year, Chhattisgarh produced 78.22 lakh tonnes of rice, with procurement centres purchasing over 95% of the output for public distribution (The Hindu Business Line, 2024) [13]. Production reached a record 9,810 thousand tonnes in 2023, and productivity improved to 2,319 kg/ha, reflecting advancements in agronomic practices and adoption of improved varieties (CEIC Data, 2023a; CEIC Data, 2023b) [1, 2].

Despite these gains, rice productivity remains under persistent threat from insect pests, among which the brown planthopper (BPH), *Nilaparvata lugens* (Stål), is the most devastating. BPH is a phloem-feeding pest that causes both direct and indirect yield losses. Directly, its feeding causes hopper burn, leading to complete wilting of rice plants. Indirectly, it transmits viral diseases such as grassy stunt and ragged stunt, compounding the yield losses. In severe outbreaks, crop losses may reach 60–100% depending on the variety and agro-ecological conditions (Padala *et al.*, 2024) [10]. Historically, BPH epidemics have

devastated rice crops across Asia, and climate change continues to exacerbate its impact. Elevated CO₂ levels and rising temperatures enhance BPH fecundity and feeding efficiency, thereby increasing its potential to cause yield losses (Chander *et al.*, 2016) [3].

Management of BPH has traditionally relied on chemical insecticides. However, indiscriminate pesticide use has led to multiple problems including resistance development in pest populations, resurgence of BPH after sprays, mortality of natural enemies, and adverse effects on environment and human health. Consequently, breeding and deployment of resistant varieties has emerged as the most eco-friendly, cost-effective, and sustainable strategy for BPH management. The resistant variety Ptb33 has been widely used as a donor in rice breeding programs, while TN1 continues to serve as a susceptible check in screening assays.

Over the past decades, extensive efforts have been made to identify resistance genes (Bph1 to Bph45) and map them using molecular markers. Recent advances in genomics and molecular breeding have facilitated the discovery of novel QTLs and resistance genes, enabling marker-assisted selection (Shi *et al.*, 2023) [12]. For instance, Govindharaj *et al.* (2025) [6] identified new resistance genes through genomic dissection of indigenous genotypes, underscoring the importance of integrating molecular tools with conventional breeding. Furthermore, biochemical studies show that resistance is often associated with antixenosis and antibiosis traits. Phenolic compounds, silica content, and tannins play critical roles in reducing BPH infestation by either deterring feeding or decreasing digestibility (Sharma *et al.*, 2024) [11].

At the same time, biofortified rice lines were developed to improve nutritional quality, such as enhanced iron, zinc, and protein levels, represent an invaluable genetic resource. These lines, while primarily bred for nutritional traits, must also be resilient to biotic stresses like BPH to ensure their adoption in farmers' fields. However, systematic screening of advanced biofortified rice lines against BPH under controlled conditions remains limited, creating a crucial research gap.

Materials and methods

Collection of Plant Material:

The experimental material consisted of thirty rice advanced biofortified lines along with standard susceptible check TN1 and resistant check Ptb-33 obtained from the Department of Molecular Biology and Biotechnology, IGKV, Raipur. NQ-BIO-1 NQ-BIO-2 NQ-BIO-3 NQ- BIO-4 NQ-BIO-5 NQ-BIO-6 NQ-BIO-7 NQ-BIO-8 NQ-BIO-9 NQ-BIO-10 NQ-BIO-11 NQ- BIO-12 NQ-BIO-13 NQ-BIO-14 NQ-BIO-15 NQ-BIO-16 NQ-BIO-17 NQ-BIO-18 NQ-BIO- 19 NQ-

BIO-20 NQ-BIO-21 NQ-BIO-22 NQ-BIO-23 NQ-BIO-24 NQ-BIO-25 NQ-BIO-26 NQ-BIO-27 NQ-BIO-28 NQ-BIO-29 NQ-BIO-30 and PTB33(Resistant Check), TN1 (Susceptible Check).

Mass Rearing of Brown Planthopper

The rearing procedure followed the protocol of Pathak and Khush. The susceptible rice cultivar *Taichung Native 1* (TN1) was used for rearing. BPH adults were initially collected from rice fields at the College of Agriculture, IGKV, Raipur, Chhattisgarh.

Screening of Biofortified Rice Lines Against BPH Insects

Thirty advanced biofortified rice lines were evaluated for their resistance to BPH using the Standard Seed Box Screening Technique (SSST) under Glass house conditions. Heinrichs *et al.*, (1985) [7].

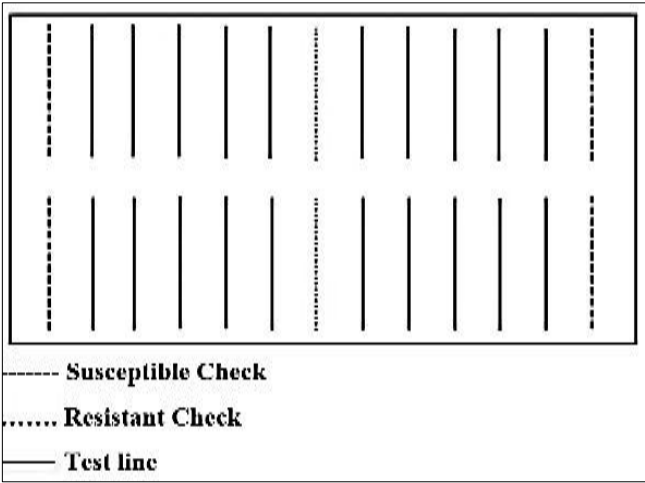


Fig 1: Layout of Standard Seed box Screening Test (SSST).

Infestation by insect on paddy

At the 15-day stage (three-leaf stage), 2nd and 3rd instar BPH nymphs were released at a density of 6–7 per seedling over the trays. The trays were rotated by 180° at regular intervals to minimize positional effects. Observations continued until the susceptible check (TN1) seedlings showed 90% mortality.

Assessment of damage causes by BPH on biofortified rice lines

Seedling reactions to BPH infestation were scored according to the Standard Evaluation System (SES) developed by IRRI (2014). A 0–9 scoring scale was used, where a score of 0 indicated highly resistant plants and 9 indicated complete mortality.

Table 3.1: Damage scale (0–9) for BPH screening (IRRI, 2014).

Score	Symptoms	Reaction
0	No visible damage	Highly resistant
1	Partial yellowing of first leaf	Resistant
3	First and second leaves partially yellow	Moderately resistant
5	Pronounced yellowing and stunted growth	Moderately susceptible
7	More than half of the plants wilted/died, remaining severely stunted	Susceptible
9	All plants dead	Highly susceptible

Table 3.2: Categorization of BPH resistance levels based on SES scale (IRRI, 2014).

Damage percentage	Score	Reaction
0-10%	0-1	Highly resistant
10-30%	1-3	Resistant
30-50%	3-5	Moderately resistant
50-70%	5-7	Moderately
> 70%	7-9	Susceptible
> 90%	9	Highly susceptible

Results and Discussion

The average plant damage scores for various rice lines, including the susceptible and resistant checks, varied from 1.8 to 9.00. The susceptible check, TN1, had an average damage score of 9.00, while the resistant check, Ptb33, recorded a score of 1.7.

In present investigation the average plant damage scores for resistant rice lines ranged from 1.8 to 2.9, as shown in Table 1. The lines NQ-BIO 8 had the lowest damage score of 1.8. was recorded NQ-BIO 10 (2.1), NQ-BIO 26 (2.6), NQ-BIO 9 (2.8) and NQ-BIO 21 (2.9) as compared to resistant check Ptb33.

In case of average plant damage scores for moderately resistant rice lines ranged from 3.2 to 5.0, as shown in Table 2. The lines NQ-BIO 6 had the lowest damage score of 3.2. This was followed by NQ-BIO 7, which shared a score of 3.3. Among all moderately resistant rice lines, NQ-BIO 15 had the highest damage score 5.0, followed by NQ-BIO 24 (4.1), NQ-BIO 25 (4.6).

The average plant damage scores for moderately susceptible rice lines ranged from 5.2 to 6.9, as shown in Table 3. Among these lines NQ-BIO 1, the lowest damage score of 5.2. while NQ- BIO 22 had a score of 5.8, and NQ-BIO 27 had a score of 6.0. The highest plant damage score among moderately susceptible rice lines was observed in NQ-BIO 17 (score 6.9).

The average plant damage scores for susceptible and highly susceptible rice lines ranged from 7.3 to 9.00, as shown in Table 4. The lowest score, 7.3, was recorded by NQ-BIO 13, while NQ-BIO 11 and NQ-BIO 19 had a score of 7.6 followed by NQ-BIO 18 (8.1), NQ-BIO 16 (8.6). The remaining 11 lines all had a damage score of 9.00.

“Phenotypic evaluation of Ptb33 introgressed rice lines” Logeshwari *et al.* (2024) ^[9] evaluated introgressed lines from Ptb33 and found several entries with strong resistance to BPH under seedbox screening and other tests. Similarly, in Dewi *et al.* (2025) ^[5], a study on doubled-haploid rice genotypes also found that among 16 genotypes, several DH lines showed resistance traits comparable to Ptb33, supporting your identification of resistant biofortified lines.

Table 1: Average plant damage score of resistant BPH rice lines.

S. No.	Name of lines (code)	Average plant damage score	Rating
1	NQ-BIO 8	1.8	R
2	NQ-BIO 10	2.1	R
3	NQ-BIO 26	2.6	R
4	NQ-BIO 9	2.8	R
5	NQ-BIO 21	2.9	R
6	Ptb 33 (Resistant check)	1.7	R
7	TN 1 (Susceptible check)	9.00	HS

* Average plant damage score based on 4 replications, R – Resistant.

Table 2: Average plant damage score of moderately BPH-resistant rice lines.

S. No.	Name of lines (code)	Average plant damage score	Rating
1	NQ-BIO 6	3.2	MR
2	NQ-BIO 7	3.3	MR
3	NQ-BIO 24	4.1	MR
4	NQ-BIO 25	4.6	MR
5	NQ-BIO 15	5.0	MR
6	Ptb 33 (Resistant check)	1.7	MR
7	TN 1 (Susceptible check)	9.00	HS

* Average plant damage score based on 4 replications, MR – Moderate resistant.

Table 3: Average plant damage score of moderately BPH-susceptible rice lines.

S. No.	Name of lines (code)	Average plant damage score	Rating
1	NQ-BIO 6	3.2	MR
2	NQ-BIO 7	3.3	MR
3	NQ-BIO 24	4.1	MR
4	NQ-BIO 25	4.6	MR
5	NQ-BIO 15	5.0	MR
6	Ptb 33 (Resistant check)	1.7	MR
7	TN 1 (Susceptible check)	9.00	HS

* Average plant damage score based on 4 replications, MS – Moderately susceptible

Table 4: Average plant damage score of BPH-susceptible and highly BPH- susceptible rice lines.

S. No.	Name of lines (Code)	Average plant damage score	Rating
1	NQ-BIO 13	7.3	S
2	NQ-BIO 11	7.6	S
3	NQ-BIO 19	7.6	S

4	NQ-BIO 18	8.1	S
5	NQ-BIO 16	8.6	S
6	NQ-BIO 2	9.0	HS
7	NQ-BIO 3	9.0	HS
8	NQ-BIO 4	9.0	HS
9	NQ-BIO 5	9.0	HS
10	NQ-BIO 12	9.0	HS
11	NQ-BIO 14	9.0	HS
12	NQ-BIO 20	9.0	HS
13	NQ-BIO 23	9.0	HS
14	NQ-BIO 28	9.0	HS
15	NQ-BIO 29	9.0	HS
16	NQ-BIO 30	9.0	HS
17	Ptb 33 (Resistant check)	1.7	MR
18	TN 1 (Susceptible check)	9.00	HS

* Average plant damage score based on 4 replications, S–Susceptible, HS–Highly susceptible

Conclusion

In present investigation 5 lines were recorded under damage score 1-3 were designated as resistant lines, whereas 5 lines were recorded under damage score 3-5 were exhibited as moderately resistant and 4 lines under damage score 5-7 were exhibited as moderately susceptible and the remaining 16 lines under damage score 7-9 were either susceptible or highly susceptible to the brown planthopper.

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